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Ileal Interposition Surgery Improves Glucose and Lipid Metabolism and Delays Diabetes Onset in the UCD-T2DM Rat

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See editorial on page 2224.

BACKGROUND & AIMS: Bariatric surgery has been shown to reverse type 2 diabetes; however, mechanisms by which this occurs remain undefined. Ileal interposition (IT) is a surgical model that isolates the effects of increasing delivery of unabsorbed nutrients to the lower gastrointestinal tract. In this study we investigated effects of IT surgery on glucose tolerance and diabetes onset in UCD-T2DM (University of California at Davis type 2 diabetes mellitus) rats, a polygenic obese animal model of type 2 diabetes. **METHODS:** IT or sham surgery was performed on 4-month-old male UCD-T2DM rats. All animals underwent oral glucose tolerance testing (OGTT). A subset was killed 2 months after surgery for tissue analyses. The remainder was followed until diabetes onset and underwent oral fat tolerance testing (OFTT). **RESULTS:** IT surgery delayed diabetes onset by 120 ± 49 days compared with sham surgery ($P < .05$) without a difference in body weight. During OGTT, IT-operated animals exhibited lower plasma glucose excursions ($P < .05$), improved early insulin secretion ($P < .01$), and 3-fold larger plasma glucagon-like peptide-1(7–36) (GLP-$1_{7–36}$) excursions ($P < .001$), and no difference in glucose-dependent insulinotropic polypeptide responses compared with sham-operated animals. Total plasma peptide YY (PYY) excursions during OFTT were 3-fold larger in IT-operated animals ($P < .01$). IT-operated animals exhibited lower adiposity ($P < .05$), smaller adipocyte size ($P < .05$), 25% less ectopic lipid deposition, lower circulating lipids, and greater pancreatic insulin content compared with sham-operated animals ($P < .05$). **CONCLUSIONS: IT surgery delays the onset of diabetes in UCD-T2DM rats which may be related to increased nutrient-stimulated secretion of GLP-$1_{7–36}$ and PYY and improvements of insulin sensitivity, β-cell function, and lipid metabolism.**

Keywords: Bariatric Surgery; Diabetes Prevention; Glucagon-Like Peptide-1; Peptide-YY.

Bariatric surgery, such as Roux-en-Y gastric bypass (RYGB), is currently the most effective long-term treatment of obesity[1,2] and has been shown to markedly improve glucose homeostasis[3–5]; however, the mechanisms by which this occurs remain undefined. The improvement of glucose homeostasis after bariatric surgery has been attributed to weight loss resulting from a reduction in gastric volume and/or reduced nutrient absorption, depending on the type of surgery. However, observations made in a number of clinical studies support a key role for endocrine changes in the reversal of type 2 diabetes after bariatric surgery. First, in patients with type 2 diabetes undergoing bariatric surgery, such as RYGB, glucose normalization often occurs before substantial weight loss.[5,6] Second, bariatric surgeries involving bypass of the proximal small intestine and/or biliopancreatic diversion are often more effective at improving obesity and reversing type 2 diabetes than bariatric surgeries involving only gastric restriction.[5,7]

These observations have lead to the development of the "hindgut" hypothesis that postulates that increased flux of unabsorbed nutrients in the distal small intestine results in the activation of a neuroendocrine negative feedback mechanism, often termed the "ileal brake," which involves increased secretion of peptides, including glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) from L cells located in the distal gastrointestinal tract.[8] Increased secretion of these hormones may contribute to weight loss and improved glucose metabolism.[8] GLP-$1_{7–36}$ (active form) acts to potentiate glucose-induced insulin secretion, inhibit glucagon secretion, decrease food intake, improve insulin sensitivity, and may also promote

Abbreviations used in this paper: ANOVA, analysis of variance; AUC, area under the curve; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; GLP-1R, glucagon-like peptide-1 receptor; IT, ileal interposition; NPY2R, neuropeptide Y receptor type 2; OGTT, oral glucose tolerance test; PCR, polymerase chain reaction; PYY, peptide YY; RIA, radioimmunoassay; RM, repeated measures; RYGB, Roux-en-Y gastric bypass; TG, triglycerides; UCD-T2DM, University of California at Davis type 2 diabetes mellitus.

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β-cell proliferation.[9] PYY_{3-36} (active form) acts to inhibit food intake and slow gastric motility and thus maintains weight loss.[10,11] Furthermore, elevations of these hormones after bariatric surgery have been reported in a number of clinical studies.[7,12]

RYGB surgery results in increased delivery of unabsorbed nutrients to the distal small intestine, but it also involves gastric restriction and bypass of the duodenum. Ileal interposition (IT) is a surgical procedure in which a segment of ileum is inserted into the proximal small intestine and provides a surgical model whereby the effect of RYGB surgery to increase the flux of unabsorbed nutrients to the distal gastrointestinal tract can be isolated from gastric restriction and duodenal bypass. IT surgery has been shown to induce weight loss and improve insulin signaling in obese and diabetic animal models.[13–16] The only diabetic animal models tested to date are the Goto-kakizaki rat and streptozotocin-treated Long–Evans rats, both of which show diabetes with a pathophysiology unlike that observed in clinical type 2 diabetes. Goto-kakizaki rats are not obese and insulin resistant.[14] Thus, diabetes in this model depends on impaired islet function, making the pathophysiology of diabetes in these animals more similar to type 1 diabetes. Similarly, streptozotocin-treated rats are considered to be a model of type 1 diabetes because hyperglycemia is the result of chemically induced β-cell destruction. In this study we investigated the effects of IT surgery on diabetes onset, glucose tolerance, and several biochemical parameters in University of California at Davis type 2 diabetes mellitus (UCD-T2DM) rats, a model of type 2 diabetes combining polygenic adult-onset obesity and insulin resistance with β-cell dysfunction.[17]

Materials and Methods

Diets and Animals

Male UCD-T2DM rats were individually housed in hanging wire cages in the animal facility in the Department of Nutrition at the University of California, Davis, and maintained on a 12:12-hour light-dark cycle. At 4 months of age rats underwent sham or IT surgery. Animals were followed until at least 1 year of age to determine the time to diabetes onset (long-term group) or were killed 2 months after surgery for tissue collection (short-term group). All animals enrolled in the study after successful surgeries completed the study. Baseline body weights, measured on the day of surgery, in the long-term groups were 585 ± 11 g and 594 ± 18 g in sham (n = 9) and IT-operated animals (n = 8), respectively. Baseline body weights in the short-term groups were 598 ± 13 g and 601 ± 13 g in sham (n = 10) and IT-operated animals (n = 10), respectively. All animals received ground chow (no. 5012; Ralston Purina, Belmont, CA). Food intake and body weight were measured 3 times a week. Nonfasting blood glucose was measured weekly with a glucose meter (One-Touch Ultra; LifeScan, Milpitas, CA) at 2:00–3:00 PM. Diabetes onset was defined as a nonfasted blood glucose value >11.1 mmol/L (200 mg/dL) on 2 consecutive weeks. The experimental protocols were approved by the University of California Davis Institutional Animal Care and Use Committee.

IT Surgery

IT surgery was performed as described by Koopmans and Sclafani.[18] Rats were placed on a liquid diet (Boost; Novartis, Minneapolis, MN) 3 days before surgery and for 5–7 days after surgery and received enrofloxacin (10 mg/kg subcutaneously) before and after surgery. Anesthesia was induced and maintained with isoflurane (2%–5%). A midline abdominal incision was made and a 10-cm segment of ileum 5–10 cm proximal to the ileocecal valve was isolated and transected. An anastomosis was made with the remaining ends of the ileum with the use of 7-0 silk suture (Ethicon). Next, a transection was made 5–10 cm distal to the ligament of Treitz. The isolated ileal segment was then inserted isoperistaltically. The transposed segment remained innervated and with its vasculature intact.

Sham-operated animals were treated in the same manner as the IT group. Sham surgeries were performed by making transections in the same locations as in the IT-operated animals, but bowel segments were reattached by anastomosis in their original position. The intestines remained their original length in both surgeries, but were reorganized with respect to which intestinal segments were exposed to incoming nutrients only with the IT surgery.

Oral Glucose and Oral Fat Tolerance Tests

One month after surgery, an oral glucose tolerance test (OGTT) was performed on animals from the short-term and long-term groups. Animals were deprived of food overnight and then received a 50% dextrose solution (1 g/kg of body weight) by oral gavage. Blood was collected from the tail for measurement of glucose and insulin concentrations. A second aliquot of blood was placed in tubes containing EDTA, aprotinin, and a dipeptidyl peptidase IV inhibitor and analyzed for $GLP\text{-}1_{7-36}$ and total glucose-dependent insulinotropic polypeptide (GIP). Serum glucose was measured with an enzymatic colorimetric assay for glucose (Thermo DMA, Louisville, CO). Serum insulin, plasma $GLP\text{-}1_{7-36}$, and total GIP were measured by rodent/rat specific enzyme-linked immunoabsorbent assays (Millipore, St Charles, MO). The 3 sham-operated animals that developed diabetes by 1 month after surgery were excluded from this data set to avoid potential confounding effects of hyperglycemia (no IT-operated animals had developed diabetes by this time point).

At 3.5 months after surgery, a subset of animals in the long-term study groups was deprived of food overnight and received Intralipid (Fresenius Kabi, Uppsala, Sweden) by oral gavage (1.5 g/kg of body weight of a 20% solu-

tion). Blood was collected from the tail into tubes containing EDTA and aprotinin. Total PYY was measured by rat/mouse specific radioimmunoassay (RIA; Millipore).

Monthly Fasted Hormone and Metabolite Profiles

Baseline and monthly blood samples were collected after an 8-hour fast from rats in the long-term groups into EDTA-treated tubes. Plasma was assayed for glucose, insulin, triglycerides (TG), cholesterol, leptin, adiponectin, and ghrelin. Fasting plasma samples were also collected from animals in the short-term groups on the day of killing, and bile acids were measured with the use of the Total Bile Acids Assay (Enzyme Cycling Method) kit (Diazyme, San Diego, CA). Plasma glucose, cholesterol, and TG were measured with the use of enzymatic colorimetric assays (Thermo DMA, Louisville, CO; L-type TG H kit, Wako Chemicals USA, Inc, Richmond, VA). Insulin, leptin, and adiponectin were measured with rodent/rat specific RIAs (rat leptin, mouse adiponectin; Millipore).

Tissue Collection, Tissue TG Content, and Adipocyte Size Determination

Two months after surgery, animals in the short-term groups were killed with an overdose of pentobarbital (200 mg/kg intraperitoneally) after an overnight fast. Tissues were weighed and flash frozen in liquid nitrogen and stored at −80°C. Liver, skeletal muscle, and adipose TG content were measured with the use of the method of Folch et al[19] for lipid extraction followed by spectrophotometric measurement of TG content (Thermo Electron).

Mesenteric adipocytes were isolated according to the method of Rodbell[20] as modified by Mueller et al.[21] Packed adipocytes (25 μL) were added to an Accuvette containing 20 mL of Isoton (Beckman Coulter, Fullerton, CA). The Accuvette was quickly placed on the sampling platform of the MultiSizer III and a 0.5-mL aliquot was counted and sized through a 280-μm aperture. With the use of the Multisizer 3.51 software, cells were counted and sorted into 300 size bins in a range of 12–1200 pL. The percentage of total adipocyte volume was calculated for each bin, and the maximum value was reported as the peak value.

Ileal Preproglucagon and PYY mRNA Expression

Ileal (~100 mg) samples were taken from the center of the transected segment and placed in a stabilization solution (1XTransPrep, nucleic acid purification lysis buffer; Applied Biosystems, Foster City, CA). Proteinase K and beads (SpexCertiprep, Metuchen, NJ) were added, and samples were homogenized in a GenoGrinder 2000 (SpexCertiprep). Total RNA was extracted with a 6100 Nucleic Acid PrepStation (Applied Biosystems). The Quantitect reverse transcriptase kit (QIAGEN, Valencia, CA) was used to DNase treat samples and generate cDNA. The probe and primers for preproglucagon (NM_012707, Rn01460420_g1) and PYY (NM_001034080, Rn 00562293_m1) and β-2 microglobulin (NM_012512, Rn00560865_m1) were purchased from Applied Biosystems (Foster City, CA). Each polymerase chain reaction (PCR) reaction contained a final concentration of 400 nmol/L for each primer and 80 nmol/L for the TaqMan probe and commercially available PCR Mastermix (TaqMan Universal PCR Mastermix; Applied Biosystems). The samples were amplified in an automated fluorometer (7900 HT FAST Real-Time PCR System; ABI). Final quantification was done with the use of the comparative Ct method (User Bulletin no. 2; Applied Biosystems) and is reported as relative transcription to the sham-operated group after using β-2 microglobulin to normalize the Ct values of target genes.

Nodose Ganglion GLP-1 Receptor and Y2R Protein Expression

Nodose ganglia were homogenized on ice in lysis buffer and cocktail inhibitors (3.03 g of Tris base and 12.7 mL of 0.5 mol/L EDTA dissolved in 500 mL of ddH_2O at pH 7.5; 1% Triton X-100, 1% antiphosphatase, 1% protease inhibitor, 2.87 μl/0.5 mL phenylmethanesulfonyl fluoride), centrifuged, and supernatant was collected. Protein concentration was determined with the use of the Bradford method (Bio-Rad, Hercules, CA). Protein (65 μg) was separated by electrophoresis and transferred to nitrocellulose membranes. Membranes were probed for GLP-1 receptor (GLP-1R) and neuropeptide Y receptor type 2 (NPY2R) with rabbit polyclonal anti–GLP-1R (Abcam, Cambridge, MA), goat anti–mouse NPY2R (United States Biological, Swampscott, MA), and rabbit anti–glyceraldehyde phosphate dehydrogenase (Cell Signaling Technology, Danvers, MA). Antibiotin horseradish peroxidase and goat anti–rabbit horseradish peroxidase (Cell Signaling Technology) were used as secondary antibodies. Immunoblotted proteins were detected by chemiluminescence with the use of 20× Lumi-GLO and 20× Peroxide reagents (Cell Signaling Technology). Optical densitometry of immunoreactive bands was measured with the use of Image Quant Version 5.1 (Molecular Dynamics, Piscataway, NJ).

Islet Immunohistochemistry and Pancreatic Insulin Content

Pancreas samples were collected and immunostained as previously described.[17]

Pancreatic insulin was extracted with a combination of methods by Davidson and Haist,[22] Dixit et al,[23] and Karam, and Grodsky.[24] Insulin was extracted from preweighed pancreas samples by mincing, sonicating, and then incubating samples overnight at 4°C in acid alcohol with aprotinin. Samples were centrifuged, the supernatant was collected, and the pellet was washed once more with the same solution. An alcohol diethyl ether solution (38% alcohol, 62% ether) was added to the supernatants and incubated overnight at 4°C. Samples were centrifuged, and the pellet was reconstituted in 0.01N hydrochloric acid and assayed for insulin content by RIA (Millipore).

Statistics and Data Analysis

Data are presented as mean ± standard error of the mean. All statistical analyses were performed with GraphPad Prism 4.00 for Windows (GraphPad Software, San Diego, CA) except for 3-factor analysis of variances (ANOVAs) that were performed with SAS 9.1 (SAS Institute, Cary, NC). OGTT and oral fat tolerance test (OFTT) data were compared by 2-factor (time × treatment) repeated measures (RM) ANOVA followed by post hoc analysis with Bonferroni's multiple comparison test. Absolute changes of body weight, food intake, and monthly circulating hormone and metabolite data were analyzed by mixed procedures 3-factor (time, treatment, disease-free days) RM ANOVA. Animals were divided into tertiles, based on disease-free days after surgery: 0–205, 206–250, ≥251 days. The incidence of diabetes was analyzed by log-rank testing of Kaplan–Meier survival curves. The age of diabetes onset, tissue weights, tissue TG content, ileal mRNA levels, and nodose ganglia protein levels were analyzed by the Student's *t* test. Differences were considered significant at $P < .05$.

Results

IT Surgery Delays the Onset of Diabetes Without Reducing Body Weight

IT surgery delayed the onset of type 2 diabetes by 120 ± 49 days compared with sham-operated animals (average age of onset, 265 ± 19 days for the sham group; 385 ± 49 days for the IT group; $P < .05$). IT surgery also reduced the incidence of diabetes such that by 1 year of age 78% of sham-operated animals were diabetic, whereas only 38% of IT-operated animals were diabetic (Figure 1*A*) ($P < .05$). This delay in onset was clearly reflected in monthly fasting plasma glucose concentrations that were 54% ± 8% higher in sham-operated animals at 8 months after surgery ($P < .05$; Figure 1*B*).

Food intake remained similar between sham- and IT-operated animals during the first 5 months after surgery (Figure 1*C*) when diabetes incidence was low. Six months after surgery food intake began to increase in sham-operated animals due to increased incidence of diabetes and development of diabetic hyperphagia (disease-free

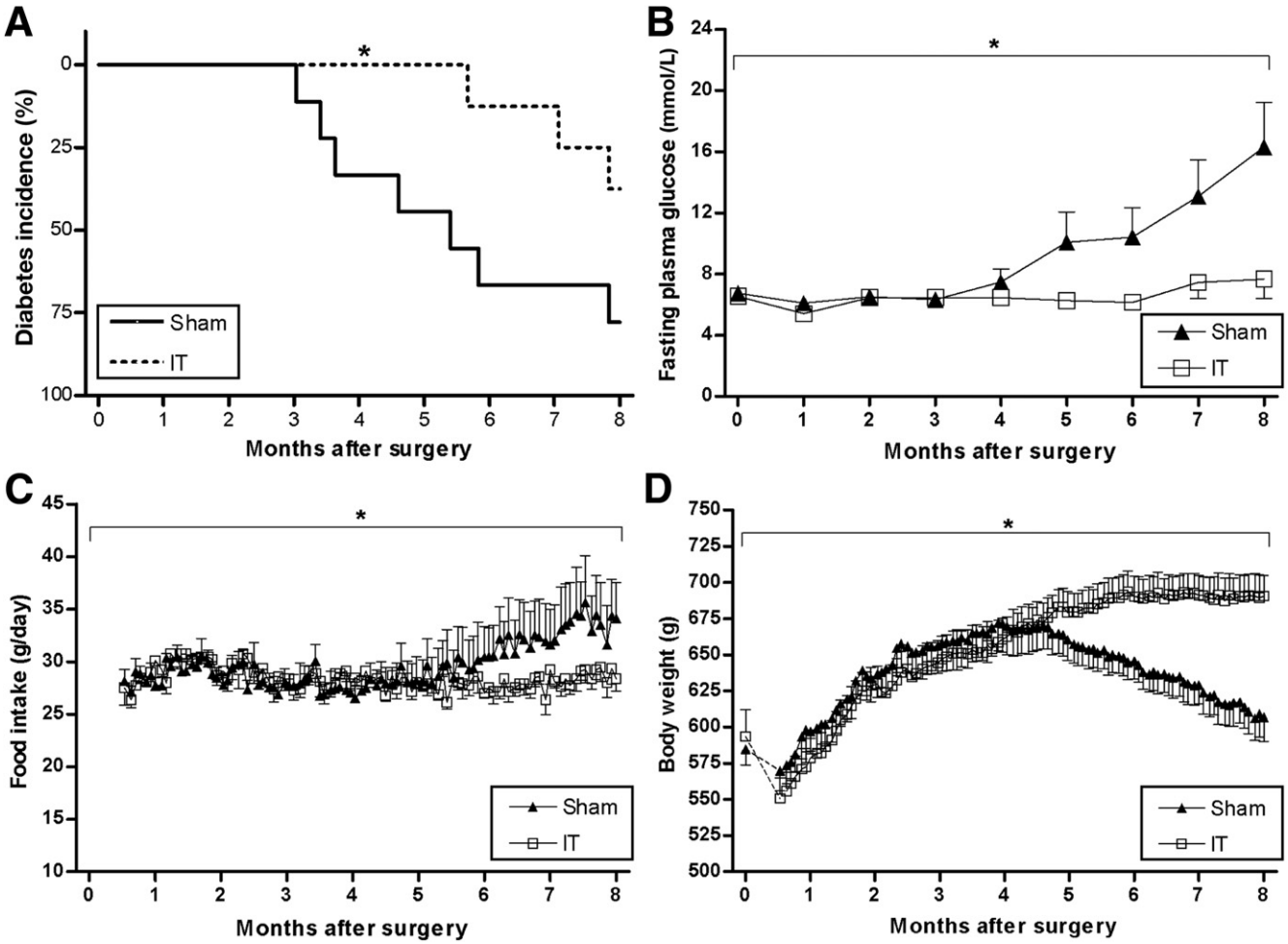

Figure 1. Kaplan–Meier analysis of diabetes incidence in sham-operated (n = 9) and IT-operated (n = 8) animals (*A*). *$P < .05$ by log-rank test. Fasting plasma glucose concentrations (*B*), food intake (*C*), and body weight (*D*) in sham-operated (n = 9) and IT-operated (n = 8) animals. *$P < .05$ disease × time by mixed procedures 3-factor (time, treatment, and disease-free days) RM ANOVA. Values are expressed as mean ± standard error of the mean.

days × time, $P < .05$; 3-factor RM ANOVA) but not an effect of treatment (treatment × time, $P = .20$; 3-factor RM ANOVA). Body weight was similar between groups until 6 months after surgery when sham-operated animals began to lose weight due to increased incidence of diabetes (disease-free days × time, $P < .05$; 3-factor RM ANOVA) but not an effect of treatment (treatment × time, $P = .11$; 3-factor RM ANOVA; Figure 1*D*).

IT Surgery Improves Glucose Tolerance and Insulin Secretion and Increases Nutrient-Stimulated GLP-1 and PYY Secretion

Glucose excursions in response to oral glucose administration were lower in prediabetic IT-operated animals than in prediabetic sham-operated animals (glucose area under the curve [AUC]: sham, 1587 ± 96 mmol/L × 180 minutes; IT, 1324 ± 53 mmol/L × 180 minutes; $P < .05$; Figure 2*A*). Fasting serum insulin concentrations were 45% ± 5% lower ($P < .01$), and the insulin AUC was 34% ± 5% lower in IT-operated than in sham-operated animals (insulin AUC: sham, 74,423 ± 7359 pmol/L × 180 minutes; IT, 49,128 ± 3352 pmol/L × 180 minutes; $P < .01$; Figure 2*B*). Furthermore, the percentage increase of plasma insulin concentrations from baseline to 30 minutes after glucose administration was 314% ± 26% in IT-operated animals and 201% ± 13% in sham-operated animals ($P < .01$), indicating improvement of glucose-stimulated insulin secretion with IT surgery. Peak GLP-1_{7-36} secretion was 3-fold higher in IT-operated animals than in sham-operated animals (GLP-1_{7-36} AUC: sham, 166 ±20 pmol/L × 60 minutes; IT, 361 ± 40 pmol/L × 60 minutes; $P < .001$; Figure 2*C*). However, ileal preproglucagon mRNA levels at 2 months after surgery did not differ between groups (Figure 2*E*). Circulating GIP concentrations did not differ between groups (GIP AUC: sham, 2740 ± 148 pmol/L × 60 minutes; IT, 2347 ± 105 pmol/L × 60 minutes; Figure 2*D*).

Peak PYY excursions in response to an oral lipid load were 3-fold higher in IT-operated animals than in sham-operated animals (PYY AUC: sham, 14,264 ± 2572 pg/mL × 180 minutes, IT, 36,895 ± 6011 pg/mL × 180 minutes; $P < .01$; Figure 3*A*). Furthermore, fasting plasma PYY concentrations were 8-fold higher in IT-operated animals than in sham-operated animals ($P < .05$). Ileal PYY mRNA content was 2-fold higher in IT-operated animals than in sham-operated animals (Figure 3*B*).

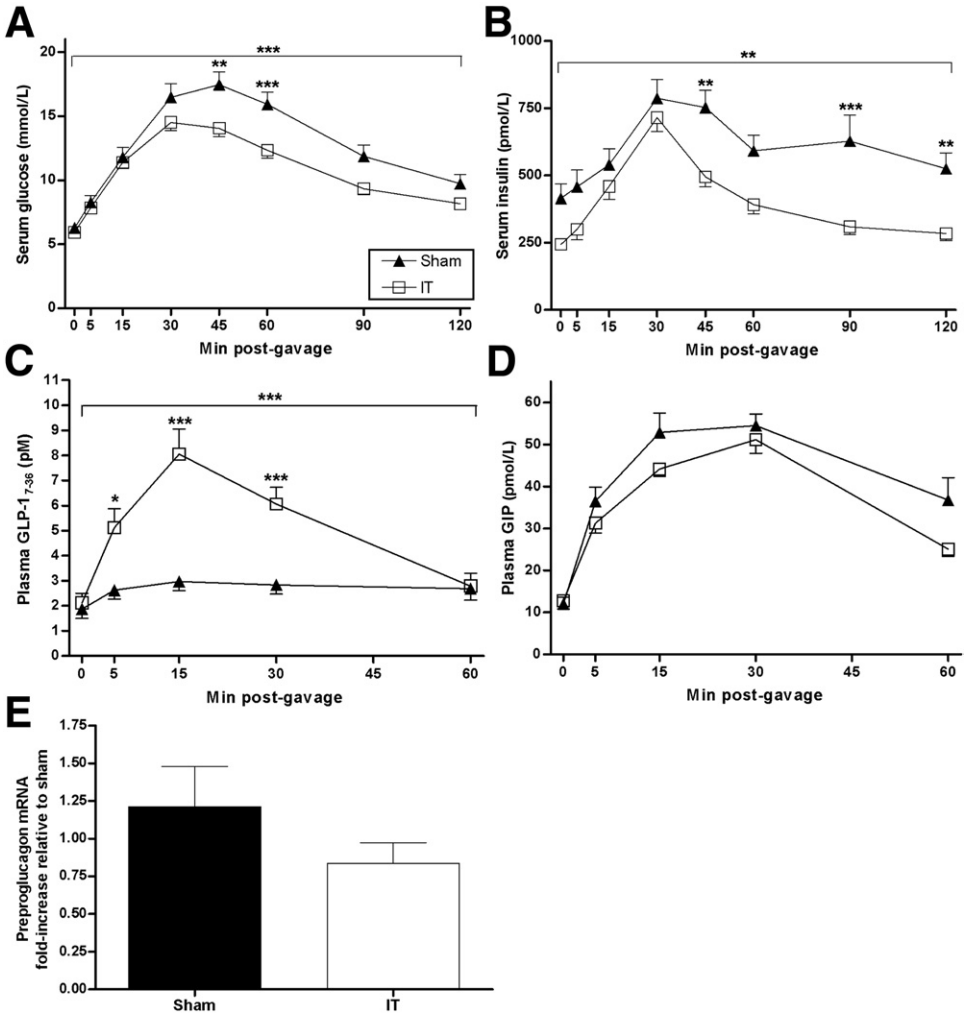

Figure 2. Serum glucose (*A*), serum insulin (*B*), plasma GLP-1_{7-36}, (*C*) and plasma GIP (*D*) concentrations after an oral glucose gavage (1 g/kg of body weight, 50% dextrose solution) in sham- (n = 15) and IT-operated (n = 18) animals at 1 month after surgery. ***$P < .0001$, **$P < .001$ by 2-factor (time × treatment) ANOVA; *$P < .05$, **$P < .01$, ***$P < .001$ by Bonferroni's posttest. Ileal preproglucagon mRNA expression 2 months after surgery in sham- (n = 10) and IT-operated (n = 7) animals (*E*). Values are expressed as mean ± standard error of the mean.

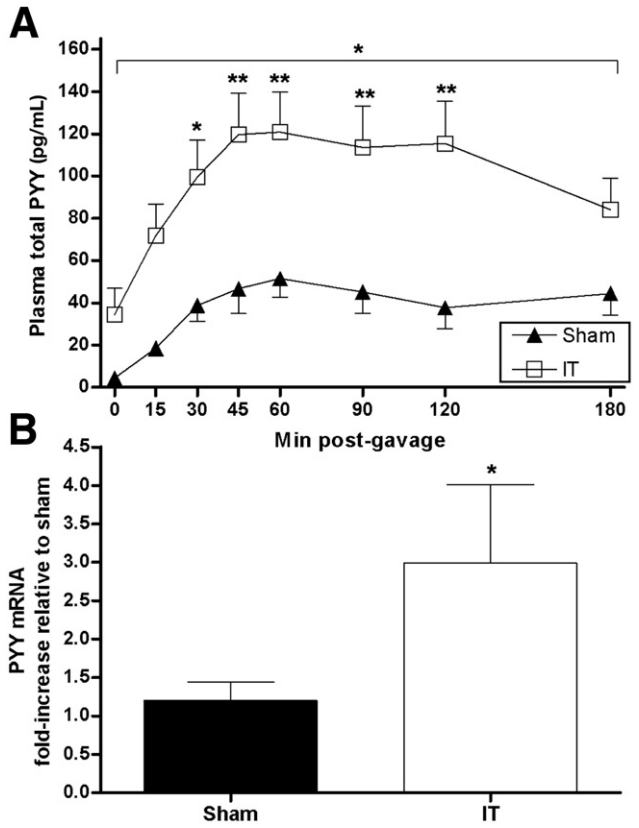

Figure 3. Plasma total PYY concentrations after an oral intralipid gavage (1.5 g/kg of body weight, 20% lipid solution) at 3.5 months after surgery in sham- (n = 7) and IT-operated (n = 7) animals (*A*). *$P < .05$ by 2-factor (time × treatment) ANOVA; *$P < .05$, **$P < .01$ by Bonferroni's posttest. Ileal PYY mRNA expression 2 months after surgery in sham- (n = 10) and IT-operated (n = 7) animals (*B*). *$P < .05$ by Student's *t* test. Values are expressed as mean ± standard error of the mean.

In contrast to the observed changes in postprandial GLP-1_{7-36} and PYY secretion, GLP-1R and NPY2R protein content in the nodose ganglion did not differ between sham- and IT-operated animals (Supplementary Figure 1).

Table 1. Tissue Weights, Tissue TG Content, and Pancreatic Insulin Content

	Sham (n = 10)	IT (n = 10)
Body weight, (*g*)	626 ± 19	599 ± 19
Epididymal fat depots (*g*)	8.9 ± 0.5	6.7 ± 0.8*
Retroperitoneal fat depots (*g*)	12.0 ± 0.8	8.8 ± 1.2*
Subcutaneous depot (*g*)	45 ± 4	38 ± 4
Mesenteric depot (*g*)	9.9 ± 0.5	8.6 ± 0.8
Total white adipose tissue (*g*)	76 ± 5	62 ± 7
Heart (*g*)	1.5 ± 0.1	1.5 ± 0.1
Kidney (*g*)	1.8 ± 0.1	1.7 ± 0.1
Liver (*g*)	21 ± 1	20 ± 1
Liver TG content (*mg/g*)	28 ± 2	21 ± 3*
Skeletal muscle TG content (*mg/g*)	5.0 ± 0.7	3.7 ± 0.3*
Inguinal TG content (%)	67 ± 2	66 ± 3
Mesenteric TG content (%)	66 ± 3	54 ± 5*
Pancreatic insulin content (*μg/g*)	5.8 ± 1.7	14.2 ± 4.1*

NOTE. Values are mean ± standard error of the mean.
*$P < .05$ compared with sham by Student's *t* test.

IT Surgery Improves Islet Structure and Increases Pancreatic Insulin Content

Figure 4 shows representative images of pancreas sections from prediabetic IT- and sham-operated animals 2 months after surgery. Islets from IT-operated animals (Figure 4*B*, *D*, and *F*) appeared more densely stained for insulin with better preservation of islet architecture than islets from sham-operated animals (Figure 4*A*, *C*, and *E*). Furthermore, pancreatic insulin content was 2.5-fold higher in IT- than in sham-operated animals ($P < .05$) (Table 1).

IT Surgery Improves Circulating Lipids and Insulin but Does Not Affect Adiponectin

Fasting plasma TG concentrations were significantly lower in IT-operated animals than in sham-operated animals due to increased diabetes incidence in the sham-operated group (disease-free days × time, $P < .01$; 3-factor RM ANOVA) and possibly due to an effect of

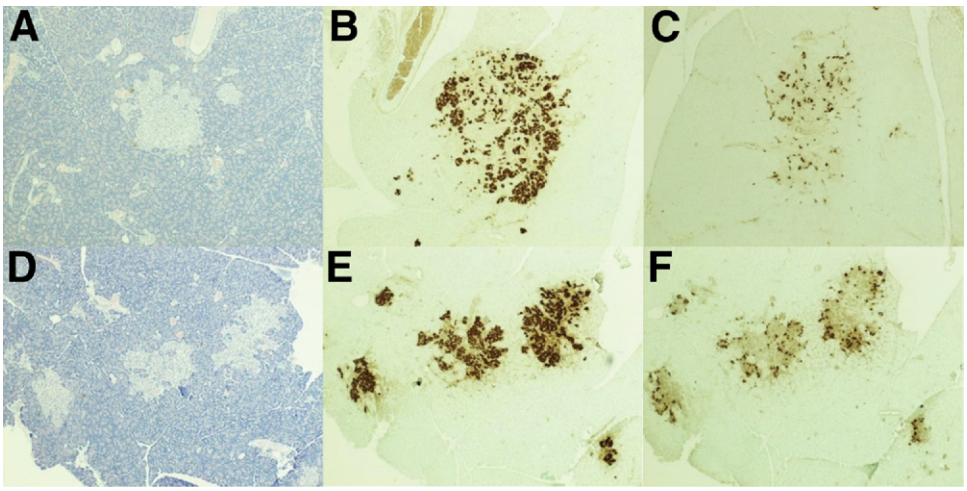

Figure 4. Representative images of pancreas sections from prediabetic sham- and IT-operated animals at 2 months after surgery. H&E stain of pancreas sections from sham-operated (*A*) and IT-operated (*D*) animals. Anti-insulin immunostaining of pancreas sections from sham-operated (*B*) and IT-operated (*E*) animals. Antiglucagon immunostaining of pancreas sections from sham- (*C*) and IT-operated (*F*) animals.

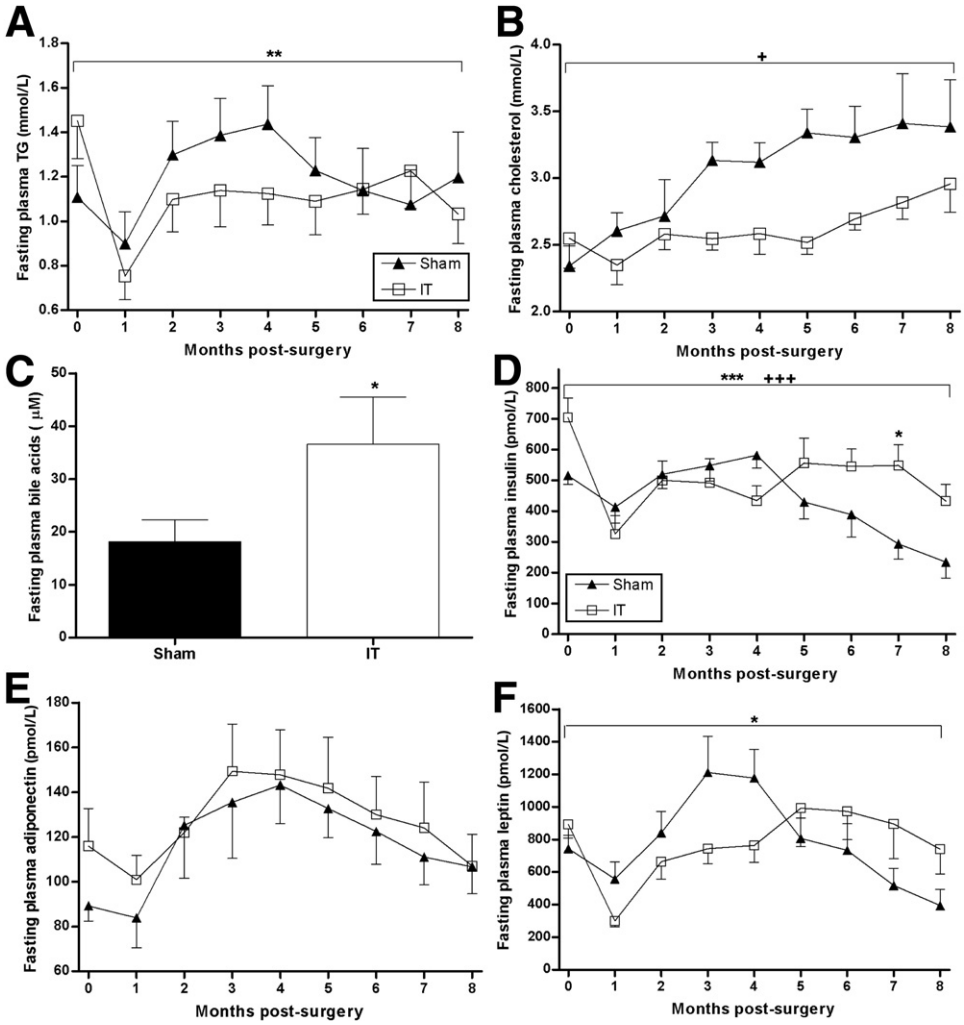

Figure 5. Monthly measurements of fasting plasma TG (*A*), cholesterol (*B*), insulin (*D*), adiponectin (*E*), and leptin (*F*) concentrations in sham- (n = 9) and IT-operated (n = 8) animals. $^{+}P < .05$, $^{+++}P < .001$ treatment × time; $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$ disease × time by mixed procedures 3-factor (time, treatment, and disease-free days) RM ANOVA. Fasting plasma bile acids at 2 months after surgery in sham- (n = 10) and IT-operated (n = 10) animals (C). $^{*}P < .05$ by Student's *t* test. Values are expressed as mean ± standard error of the mean.

treatment (treatment × time, $P = .08$; 3-factor RM ANOVA) (Figure 5*A*). Fasting plasma cholesterol concentrations were significantly lower in IT-operated than in sham-operated animals due to an effect of treatment (treatment × time, $P < .05$; 3-factor RM ANOVA) and not an effect of diabetes incidence (disease-free days × time, $P = .17$; 3-factor RM ANOVA) (Figure 5*B*). Fasting plasma bile acid concentrations were 2 times higher in IT-operated than in sham-operated animals at 2 months after surgery ($P < .05$) (Figure 5*C*).

Fasting plasma insulin concentrations remained stable in the IT-operated group, whereas fasting plasma insulin concentrations decreased starting at 5 months after surgery in the sham-operated animals due to both an effect of treatment (treatment × time, $P < .001$; 3-factor RM ANOVA) and diabetes incidence (disease-free days × time, $P < .001$; 3-factor RM ANOVA) (Figure 5*D*). Fasting plasma adiponectin concentrations did not differ between groups (Figure 5*E*). Plasma leptin concentrations were significantly lower in IT-operated than in sham-operated animals due to an effect of diabetes incidence (disease-free days × time, $P < .05$; 3-factor RM ANOVA) but not an effect of treatment (treatment × time, $P = .41$; 3-factor RM ANOVA) (Figure 5*F*).

IT Surgery Decreases Adipocyte Size and Tissue TG Content

Despite comparable body weight at 2 months after surgery, IT-operated animals had smaller epididymal and retroperitoneal adipose depots than did sham-operated animals ($P < .05$) (Table 1). Peak mesenteric (Figure 6*A* and *B*) and subcutaneous (Figure 6*C* and *D*) adipocyte volumes were ~35% smaller in IT-operated animals than in sham-operated animals ($P < .05$). Mesenteric fat depot and liver and skeletal muscle TG content were all ~25% lower in IT-operated animals than in sham-operated animals ($P < .05$) (Table 1).

Discussion

In the present study we investigated the effects of IT surgery to delay the onset of type 2 diabetes in UCD-T2DM rats. This is the first study to investigate the efficacy of IT surgery to delay the development of type 2

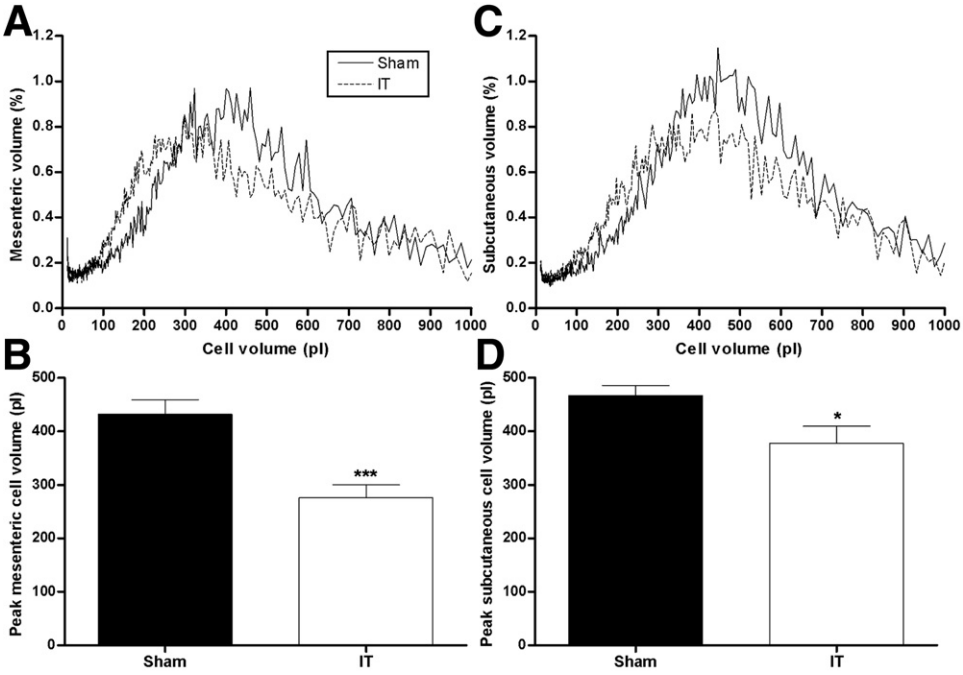

Figure 6. Mesenteric (*A*) and subcutaneous cell (*C*) volume distribution and mesenteric (*B*) and subcutaneous (*D*) peak cell volume. $^{*}P < .05$, $^{***}P < .001$ by Student's *t* test. Values are expressed as mean ± standard error of the mean.

diabetes. Given the reproducible effects of bariatric surgery to normalize glucose homeostasis and given the increasing prevalence of type 2 diabetes, the effects and mechanisms of different types of bariatric surgery to delay or prevent the development of type 2 diabetes should be investigated for the potential identification of new therapies for diabetes. In this study, IT surgery markedly delayed the onset of type 2 diabetes compared with sham surgery. The similar body weights in sham- and IT-operated animals provide support for the hypothesis that surgery-induced changes of endocrine function are likely to have an important role in the delay of diabetes onset, independent of differences in body weight. The observation that increases of nutrient-stimulated GLP-1_{7-36} and PYY secretion in IT-operated animals did not result in decreased food intake brings into question the role of endogenous GLP-1 and PYY in the regulation of food intake and suggests that postoperative increases of these hormones may not be responsible for the weight loss observed after bariatric surgeries, such as RYGB. However, the increases of nutrient-stimulated GLP-1_{7-36} secretion may have contributed to the observed improvements of islet function, insulin sensitivity, and lipid metabolism in IT-operated rats compared with sham-operated rats.

Increases of GLP-1 may have improved islet function by increasing islet glucose sensitivity and insulin secretory capacity. GLP-1 has been shown to not only potentiate glucose-stimulated insulin secretion but also to increase insulin synthesis, stimulate β-cell proliferation, and prevent β-cell apoptosis.[9,25] Improvement of glucose-stimulated insulin secretion was clearly shown at 1 month after surgery during the OGTT in which IT-operated animals exhibited 3-fold greater glucose-stimulated insulin secretion than did sham-operated animals. Furthermore, IT-operated animals had 2.5-fold greater pancreatic insulin content than did sham-operated animals.

IT-operated animals also exhibited improved insulin sensitivity compared with sham-operated animals, based on decreased fasting plasma insulin concentrations and the lower glucose and insulin excursions during the OGTT. Increases of GLP-1 may have improved insulin sensitivity by reducing glucotoxicity and lipotoxicity.[26] GLP-1 reduces glucotoxicity by improving islet function and insulin sensitivity and by reducing hepatic gluconeogenesis.[9,27] GLP-1 may contribute to reductions in lipotoxicity by stimulating fat oxidation during meals.[28] Thus, increases of nutrient-stimulated GLP-1_{7-36} release may have contributed to a reduction in adipocyte size in IT-operated animals compared with sham-operated animals by increasing lipolysis in fat cells during meals.[29] This decrease of adipocyte size may have contributed to lower ectopic and circulating TG concentrations in IT-operated animals, thereby contributing to improved insulin sensitivity. Larger adipocytes, especially in the mesenteric adipose depot, have been shown to be more insulin resistant[30] and therefore less sensitive to the antilipolytic actions of insulin, resulting in greater free fatty acid secretion into the portal vein and TG accumulation in the liver.[31] TG deposition in the liver is considered a major contributor to hepatic insulin resistance[32,33] and also promotes increased circulating TG, resulting in greater TG deposition in peripheral tissues,[34] further exacerbating systemic insulin resistance,[32] and possibly islet lipotoxicity.[35]

Changes of bile acids, which are increased after RYGB in human beings[36] and IT surgery in rodents,[15,37] may

represent another mechanism by which bariatric surgery leads to improved insulin sensitivity and resolution of diabetes. Interposition of the ileum proximally may increase absorptive capacity of the intestinal epithelium, resulting in greater bile acid reabsorption. Bile acids have been shown to increase energy expenditure, improve circulating lipid profiles and glucose homeostasis, and stimulate GLP-1 and PYY secretion.[38–41] Furthermore, simply diverting the common bile duct to the distal small intestine in streptozotocin-treated rats has been reported to completely resolve diabetes.[42] Thus, postoperative increases of bile acids may have contributed to the increases of circulating GLP-$1_{7–36}$ and PYY concentrations and the decreases of circulating TG and cholesterol concentrations.

Unlike some previous studies of IT surgery in rats,[13,16] ileal preproglucagon mRNA levels were not elevated in IT-operated animals, suggesting that increases of circulating GLP-$1_{7–36}$ concentrations after IT surgery in UCD-T2DM rats are primarily due to increased secretion. Similar to previously reported data,[16] the increase of nutrient-stimulated PYY secretion was accompanied by an increase of ileal PYY mRNA expression. Thus, increases of plasma PYY concentrations were probably due to increased synthesis and secretion.

As expected, GIP secretion did not differ between IT- and sham-operated animals because the position of the duodenum remained unchanged after both surgeries. This is in contrast to results obtained after RYGB, in which GIP secretion is often decreased, probably because of the surgical exclusion of the duodenum from contact with ingested nutrients.[7,12] Decreases of GIP have been proposed to contribute to the glucose-lowering effects of RYGB.[43] However, in this study, improvements of glucose homeostasis and a delay in diabetes onset were observed without reductions of GIP responses.

In conclusion, we have demonstrated for the first time that IT surgery can delay the onset of diabetes in an animal model of type 2 diabetes. Factors that may be involved in delaying diabetes onset include increases of plasma bile acid concentrations and nutrient-stimulated GLP-$1_{7–36}$ secretion. Increases of circulating bile acid concentrations may have contributed to the increases of GLP-$1_{7–36}$ secretion and improvements of circulating lipids. The increases of GLP-$1_{7–36}$ secretion probably contributed to improvements of insulin sensitivity, islet function, and lipid metabolism. It would be of interest to investigate why the onset of diabetes was only delayed and not prevented altogether after IT surgery. Adaptation (eg, down-regulation of GLP-1 production and secretion) in the transposed segment of distal intestine could account for this. Measurements of stimulated GLP-1 secretion and GLP-1 expression in the transposed segment at later times after surgery would be informative in this regard. Further studies of the effects of IT and other bariatric surgical procedures in the UCD-T2DM rat model will provide additional insight into the surgically induced improvements of metabolism and will identify new strategies for the prevention and treatment of type 2 diabetes.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at doi: 10.1053/j.gastro.2010.03.005.

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Conflicts of interest

The authors disclose no conflicts.

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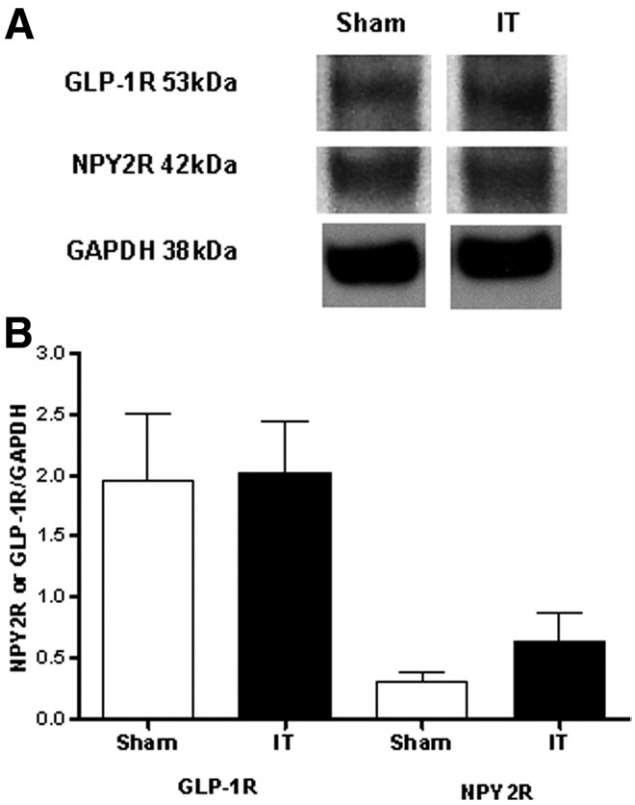

Supplemental Figure 1. Representative Western blots of nodose ganglion glucagon-like peptide-1 receptor (GLP-1R) and neuropeptide Y receptor type 2 (NPY2R) protein levels. Images presented were taken from the same gel (*A*). Quantification of nodose ganglion GLP-1R and NPY2R protein levels (*B*). Sham: n = 10, ileal interposition (IT): n = 10. Values are expressed as mean ± standard error of the mean.